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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) As part of a research project that is directed at characterizing indices of stress in military environments, we measured urinary metabolites, electrolytes and cortisol in 10 soldiers participating in the CANE 1 sustained operations exercise at Ft. Hunter-Liggett, California in April 1983. The CANE 1 (phase 1 of Combined Arms in a Nuclear/chemical Environment) exercise was conducted to assess platoon performance in units wearing MOPP gear. MOPP (Mission Oriented Protective Posture) is the individual clothing worn to protect individuals. --CONTINUED ON REVERSE SIDE--		

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20. against exposure to chemical warfare agents. MOPP includes a special suit, mask, boots and gloves.

The exercise included a baseline pretrial period of several days, a 3 day sustained operations exercise in normal field gear, an intertrial period, a second 3 day sustained operations exercise wearing MOPP gear and a posttrial period. Total urine output was collected in 12 hr blocks during each period, processed and later analyzed for various metabolic compounds, electrolytes and cortisol. The purpose of the research study was to determine whether any of the urinary indices of stress or metabolism measured would reflect the changes in the environment during the course of the exercise such that these indices could be used to objectively assess the degree of "stress" associated with each phase of the exercise.

Soldiers were found to excrete more cortisol during the sustained operations phases of the exercise than in pretrial, intertrial and posttrial periods. In addition, the daily pattern of cortisol excretion was altered in soldiers operating around-the-clock. No difference in cortisol was seen between leaders and non-leaders. Recovery to pretrial baseline values was relatively rapid. In addition, changes in urine volume, specific gravity, nitrogen and electrolyte excretion were also seen. No differences in the two field trials (one in regular field gear and one in MOPP gear) were noted. Since the weather during the first field trial was worse than the weather during the second (MOPP) field trial, the effects of weather might have balanced out the aversiveness of the MOPP gear. Also, previous experience with the scenario during the first field trial might have diminished the stress of the second trial by a training effect.

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The Effect of Sustained Field Operations on Urinary Electrolytes and Cortisol

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Urinary cortisol and electrolytes were measured in 10 soldiers participating in a sustained operations field exercise. Urine was collected in consecutive 12 hour periods during two 72 hour field trials as well as pretrial, intertrial and posttrial phases. Urine specific gravity and cortisol excretion increased markedly during both field exercise periods, while urine volume significantly decreased. The increase in cortisol excretion occurred primarily in the 12 hour night collections (1700 to 0500 hours) and disrupted the normal circadian variation in cortisol excretion.

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As part of a research project that is directed at characterizing indices of stress in military environments, we measured urinary electrolytes and cortisol in 10 soldiers participating in a sustained operations exercise. The exercise was conducted to assess platoon performance in units wearing MOPP gear. MOPP (Mission Oriented Protective Posture) is the individual clothing worn to protect individuals against exposure to chemical warfare agents. MOPP includes a special suit, mask, boots and gloves.

The exercise included a baseline pretrial period of several days, a 3 day sustained operations exercise in normal field gear, an intertrial period, a second 3 day sustained operations exercise wearing MOPP gear and a posttrial period. Total urine output was collected in 12 hr blocks during each period, processed and later analyzed for electrolytes and cortisol. The purpose of the research study was to determine whether any of the urinary indices measured would reflect the changes in the environment during the course of the exercise such that these indices could be used to objectively assess the degree of "stress" associated with each phase of the exercise.

Cortisol was measured because (1) it is responsive to stress in humans¹⁻⁴ (2) it normally varies in a regular rhythm over the course of a day and might be a sensitive marker for sleep schedule disruption during a sustained operation⁵ (3) it has important physiological functions.⁶

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Methods

Field Operations

The sustained operations exercise was designed and executed to assess performance in MOPP gear. In addition to assessments of military effectiveness as judged by results of offensive and defensive operations, physiological and psychological assessments were performed on some soldiers from each of the nine mechanized platoons that cycled through the exercise. Each platoon cycled through a similar scenario of pretrial training and baseline testing followed by a 3 day sustained operations exercise, an intertrial interval of several days, a second 3 day sustained operations exercise and finally a 2 day posttrial period. During the field operations, which included offensive and defensive operations against an opposing force, soldiers used a laser simulation system. Vehicles and personnel wore sensors that determined when a "hit" had occurred. Each soldier carried a backpack of electronic equipment that transmitted position and "hits and misses" to a central computer facility. The two field trials differed primarily in the type of clothing worn. During one trial, soldiers wore normal field gear while during the other field trial, MOPP gear was worn. The level of MOPP (I through IV) varied during the course of the 3 day sustained operations. The longest period of MOPP IV (the most protective level with gloves, boots and masks worn) lasted for 12 hours. Decontamination periods were also incorporated into the MOPP sustained operations phase.

We studied one platoon, the sixth platoon to cycle through the exercise. In the cycle we followed, the first field exercise was in regular field gear and the second 3 day sustained operations was conducted in MOPP. The weather during the first trial was cool (50 to 60°F) and very rainy.

There were problems with equipment in the mud and rain. During the second (MOPP) trial, the weather was again cool (50 to 60°F) but mostly sunny.

Subjects

During a pretrial briefing, we requested that the platoon leader, the assistant platoon leader, the 3 squad leaders and 5 soldiers in non-leadership positions participate in the study. Selection was based solely on the soldier's position in the platoon and not on any individual characteristics. The objectives of the study were presented orally and each soldier was also given a written description of the study to be performed. All 10 selected soldiers gave written informed consent for the study. The 10 soldiers were given 1 liter plastic screw-capped bottles and were instructed to carry the bottles in the leg pocket of their fatigues. They were directed to always urinate in these bottles, to transfer the contents of the bottles to separate individually labelled bottles kept nearby in a dry ice container as often as possible and to report any deviations from this procedure to one of the researchers. During the pretrial, intertrial and posttrial periods, these dry ice containers were kept in the barracks latrine. During the field exercises, the dry ice containers were moved to field locations nearby the platoon. Members of the research team were in frequent contact with the soldiers in the field to assist in transferring urine from the individual bottles to the collection bottles kept on dry ice.

Urine Processing

Each 12 hours the urine collection bottles in the dry ice containers were replaced with new bottles. The frozen urine was thawed, the volume and specific gravity measured and then separate aliquots were transferred to several test tubes for later assay procedures. The test tubes were kept in a freezer and then shipped to Walter Reed Army Medical Center on dry ice.

Assay Procedures

Urinary free cortisol was measured by radioimmunoassay.^{7,8} Briefly, 0.5 ml of urine was extracted with 2.0 ml of ethyl acetate. Aliquots of the ethyl acetate fraction were evaporated and the residue was redissolved in phosphate buff-

ered saline. Aliquots were analyzed for cortisol by radioimmunoassay, using an antibody produced in our laboratory in rabbits against cortisol-BSA conjugate. ³H-cortisol (New England Nuclear) was used as the radioactive marker. After a three hour incubation at 4°C, Somogyi reagents were used to separate free from bound hormone. The coefficients of variation for this assay are 6% for intrassay and 10% for interassay. Sodium and potassium were measured using the Beckman E-4 ISE system with Beckman reagents.

Statistics

Data were analyzed by analysis of variance (ANOVA) using BMDP statistical programs. Main effects of exercise phase (pretrial, field 1, intertrial, field 2, and posttrial), time of collection (1700 to 0500 and 0500 to 1700), and leadership (platoon leader, assistant platoon leader and three squad leaders vs the five other subjects) were examined. Followup comparisons of specific groups were made using Student's *t* test after overall significant *F* scores were found in the ANOVA.

Results

As shown in Table I, urine volume decreased and urine specific gravity increased during the two field exercises. Analysis of variance found a significant effect of exercise phase on urine volume, $F(4,220) = 5.49$, $p < 0.001$, and specific gravity, $F(4,220) = 16.85$, $p < 0.001$. The correlation between increased specific gravity and lower volumes during the field portions of the exercise coupled with our own observations lead us to conclude that the volumes collected reflect different amounts excreted rather than a lack of complete collection during the field trials.

Since volume changes during the different phases would influence the concentration of urinary constituents, the urinary cortisol levels are expressed in Table II in terms of total cortisol excreted (μg per 12 hours). Cortisol varied significantly over the different phases of the exercise, $F(4,220) = 3.70$, $p < 0.01$ for total cortisol. This effect is even greater if cortisol concentration values are compared, $F(4,220) = 13.8$, $p < 0.001$. More cortisol was excreted during the two field

TABLE I
URINE VOLUME AND SPECIFIC GRAVITY

Phase	Pretrial	Field 1	Intertrial	Field 2	Posttrial
Volume (ml)	747 \pm 55	578 \pm 42*	513 \pm 36*	495 \pm 34*	624 \pm 63
Specific Gravity	1.015 \pm .001	1.023 \pm .001*	1.017 \pm .001	1.023 \pm .001*	1.018 \pm .002
N	55	55	44	54	17

Values represent the mean \pm SEM. * Differs significantly from pretrial phase, Student's *t* test, two-tailed, $p < 0.05$. N is the total number of 12 hr collections during the indicated test phase.

exercises than during the pretrial, intertrial or posttrial periods. In addition, the daily pattern of cortisol was affected by the sustained field operations (Table III). In the pretrial period, three times as much cortisol was excreted during the 0500 to 1700 (day) period as during the 1700 to 0500 (night) collection, $F(1,220) = 34.7$, $p < 0.0001$. During the field trials, which involved round-the-clock operations, a larger proportion of the cortisol excreted was found in the night collection (1700 to 0500). In fact most of the additional cortisol excreted during the field operations was during the night collection. By the time of the second field trial, equal amounts of cortisol were excreted during the day and night collections. No differences in cortisol excretion were seen between leaders and nonleaders, $F(1,220) = 0.12$, $p > 0.05$ (Table IV).

Urinary potassium levels (Table V) were somewhat increased during the two field phases, $F(4,220) = 6.44$, $p < 0.0001$. Urinary sodium levels were lowest during the MOPP field trial possibly as a result of increased sodium loss via

sweating. However, this effect was not statistically significant, $F(4,220) = 0.71$, $p > 0.05$.

Discussion

Soldiers participating in a military exercise were found to excrete more cortisol during the sustained operations phases of the exercise than in pretrial, intertrial and posttrial periods. In addition, the daily pattern of cortisol was altered in soldiers operating around-the-clock. No differences in cortisol excretion were seen between leaders and nonleaders. Recovery to pretrial baseline values was relatively rapid. In addition, changes in urine volume, specific gravity and electrolyte excretion were also seen. No differences in the two field trials (one in regular field gear and one in MOPP gear) were noted. Since the weather during the first field trial was worse than the weather during the second (MOPP) field trial, the effects of weather might have balanced out the aversiveness of the MOPP gear. Also, previous experience with the scenario during the

TABLE II
URINARY CORTISOL

Phase	Pretrial	Field 1	Intertrial	Field 2	Posttrial
Cortisol	34.1 ± 3.9	52.0 ± 5.0*	33.7 ± 3.8	44.3 ± 4.0*	36.2 ± 4.9

Values represent the mean total cortisol ($\mu\text{g}/12 \text{ hr}$) ± SEM. * Differs significantly from pretrial phase. Student's *t* test, two-tailed, $p < 0.05$. For N, see TABLE 1.

TABLE III
DAY VS NIGHT URINARY CORTISOL

Phase	Pretrial	Field 1	Intertrial	Field 2	Posttrial
Day	54.6 ± 5.3	62.3 ± 6.3	47.6 ± 5.1	43.2 ± 5.0	48.0 ± 6.8
Night	14.4 ± 2.2	29.4 ± 4.3	23.1 ± 4.3	46.2 ± 6.9	23.1 ± 3.3

Values represent the mean cortisol in $\mu\text{g}/12 \text{ hr}$ ± SEM. Day collected 0500 to 1700. Night collected 1700 to 0500. For N, see TABLE 1.

TABLE IV
EFFECTS OF LEADERSHIP ON URINARY CORTISOL

Phase	Pretrial	Field 1	Intertrial	Field 2	Posttrial
Leaders	33.2 ± 5.7	44.4 ± 5.8	35.8 ± 5.8	44.2 ± 4.2	40.2 ± 7.5
Nonleaders	35.1 ± 5.4	62.0 ± 8.2	31.4 ± 4.7	44.4 ± 6.9	32.8 ± 6.6

Values are mean cortisol ($\mu\text{g}/12 \text{ hrs}$) ± SEM. For N, see TABLE 1.

TABLE V
URINARY ELECTROLYTES

Phase	Pretrial	Field 1	Intertrial	Field 2	Posttrial
Sodium	71 ± 7	65 ± 7	61 ± 7	56 ± 5	61 ± 14
Potassium	22 ± 2	28 ± 3	13 ± 2	20 ± 2	17 ± 2

Values for sodium and potassium are mean mEq/12 hr ± SEM.

first field trial might have diminished the stress of the second trial by a training effect.

Cortisol was found to be a useful indicator of stress and of circadian disruption in soldiers participating in a sustained field exercise.

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